The Formative Influences and Comparative Effectiveness of Various Plant Hormone-like Compounds*

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Plant physiology has gone a long way since Boysen-Jensen discovered that the stimulus which causes cell elongation and tropic curvatures in coleoptiles passed through a discontinuity of tissue and appeared to be of a chemical nature (1). Since that time many chemical compounds, natural and synthetic, have been found which when applied to plants act like hormones. In addition to cell elongation these substances cause cell division, induce new organs, prevent abscission, inhibit buds, modify the pattern of organs, and otherwise regulate the growth of plants. Such substances have been given various names as hormones, auxins, growth substances, growth promoters, growth regulators, etc. None of these designations is satisfactory because a single substance has the capacity to induce several varied responses. The word "formative" has often been used to describe the effects of hormone-like compounds on plants. This term did not seem significant until recently when it was found that some of these physiologically-active compounds have a decidedly regulating and "formative" effect on the new growths of the entire plant (5, 7, 3, 4). This is in contrast with locally induced cell elongation. The subject of this paper concerns especially formative influences and comparative activity of several hormone-like compounds which modify the pattern of leaves, flowers, and fruit and which change the correlation phenomena of organs.

METHODS AND MATERIAL

The activity of growth substances was usually detected by curvatures resulting from induced cell elongation or by formative effects on later growth. The former response occurred within a comparatively short period of time (20 to 60 minutes). Formative effects appeared in days or weeks after the plant had time to produce new organs. The first evidence of formative effects appeared on new leaves which were modified in size, shape, pattern, and texture. Later the effects appeared on flowers, fruit, growth habit, and correlation phenomena of organs.

The chemicals were applied to plants in water solution, as lanolin preparations, and as vapors. Various spreaders were used with water solutions but were generally considered not essential. Water solutions (10 to 300 mg./l.) were sprayed on the plants with a nasal atomizer, applied to the soil, and injected into the stem with a glass capillary tube. Lanolin (or other oily substance)

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preparations were made up with a series of concentrations of the chemical, ranging from 0.005 to 20.0 mg./g. of lanolin. These preparations were applied to local parts of the plant with a glass rod. To induce epinasty the material was applied to the upper side of a young leaf petiole; to induce curvature of the stem the material was applied along one side of a young stem. Chemicals active for cell elongation caused negative (away from treated side) curvatures within a short time. The same treatment also served to determine whether the chemicals had a formative influence on new growth. Plants were exposed to vapors of the various compounds under bell jars, closed greenhouses, glass cages, or other closed containers which could be kept reasonably tight. The esters were more volatile than acids or amides and were considered best for vapor treatments. The ultimate effects, however, were the same for all. The amount of ester required under the bell jar was less than 1 milligram. When heat was required to volatilize the chemical, a small amount was placed on a watch glass which in turn was placed on a warm or hot inverted crucible under a bell jar. In the greenhouse a hot plate supplied the heat and an electric fan circulated the air.

Most of the chemical compounds used in the experiments and listed in the tables were synthesized in the Boyce Thompson Institute laboratories. A few were available from commercial supply houses.

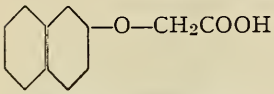
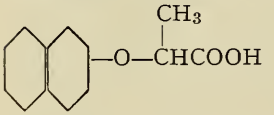
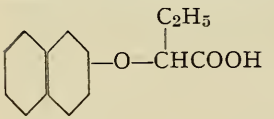
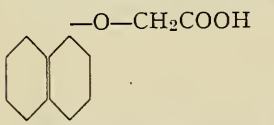
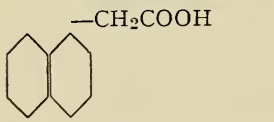
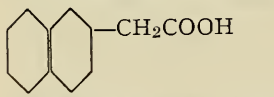
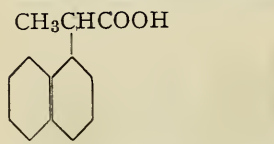
Results

For the study of formative influences three groups of compounds stand out above all others. They are β-naphthoxy acids, substituted phenoxy derivatives of the lower fatty acids, and substituted benzoic acids. The substituted groups were nitro, amino, methyl, or halogen radicals. These were used alone or in various combinations substituted in the naphthalene or benzene ring.

β-Naphthoxy compounds. β-Naphthoxyacetic acid and the higher homologs, propionic and butyric acids, were the first observed to have special formative influences (5). They were found to have in common with other plant hormone-like compounds the capacity to cause cell elongation, parthenocarpic development of ovaries, and to induce roots. Table 1 shows a list of naphthoxy and naphthalene compounds and their activity for cell elongation and formative influences.

It is interesting to note that for cell elongation naphthoxy compounds must have the chain of the molecule linked to the beta position in the ring while the alpha position is required for naphthaleneacetic acid. α-Naphthoxyacetic acid is inactive for cell elongation but has a slight formative influence. Neither α- nor β-naphthaleneacetic acid has a formative influence which modifies the pattern of leaves.

TABLE 1. Molecular Configuration and Comparative Activity of Naphthoxy and Naphthalene Compounds

Substances	Cell elongation	Modification of tomato leaves
 β—Naphthoxyacetic acid	Active	Active
 α—(β-Naphthoxy) propionic acid	Active	Slightly Active
 α—(β-Naphthoxy)-n-butyric acid	Active	Active
 α—Naphthoxyacetic acid	Inactive	Slightly Active
 α—Naphthaleneacetic acid	Active	Inactive
 β—Naphthaleneacetic acid	Inactive	Inactive
 α—(α-Naphthalene) propionic acid	Inactive	Inactive

Attention has been called to the fact that though β-naphthoxyacetic acid and its higher homologs have a formative influence on growth, there are qualitative differences in responses induced with these compounds (6). It should be pointed out also that different species bring out further qualitative differences. β-Naphthoxyacetic acid, for example, modifies the leaves of Turkish tobacco (*Nicotiana tabacum*) while β-naphthoxypropionic acid has little or no effect on the pattern of leaves of this species.

Substituted phenoxy compounds. There are many active substituted phenoxy compounds. The activity appears to be related to the kind, number, and position of substituted groups in the benzene ring. In general, halogen substitutions bring about greater activity than methyl, amino, or nitro groups. With a single halogen group substitution the para position is more effective than the ortho. However, 2,4-dichlorophenoxyacetic acid is more effective than either *o*- or *p*-chloro phenoxyacetic acid (4, 7).

Nitro group substitutions do not activate the phenoxy molecule except in the meta position. The amino group activates the molecule when substituted in the para position. The chlorine atom in the ortho, meta, or para position activates the phenoxy molecule. These comparisons are shown in table 2.

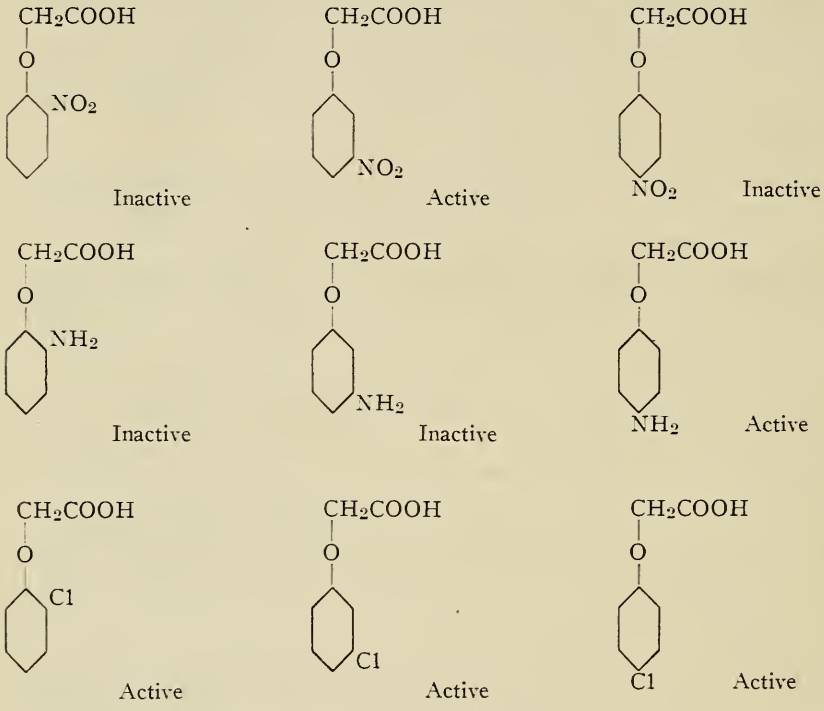
Table 3 shows the comparative activity of non-substituted and chloro-substituted phenoxy compounds. Phenoxyacetic acid does not modify the pattern of leaves though its propionic and butyric acid homologs do. This is in contrast with ortho, para, and 2,4-dichlorophenoxy compounds where the acetic acid form modifies leaves but the corresponding propionic and butyric acid homologs do not. These are in further contrast with *m*-chlorophenoxy and 2,4,5-trichlorophenoxy compounds where neither the acetic acid forms nor their higher homologs modify leaves though all are active for cell elongation. In all active phenoxy compounds the nucleus of the molecule was linked to the chain at the alpha carbon atom. Comparable beta linkages made inactive compounds.

2,4,6-Trichlorophenoxyacetic acid and the propionic acid homolog did not cause cell elongation but had a slight capacity to modify organs. 2,3,4,6-Tetrachlorophenoxyacetic acid and 2,3,4,5,6-pentachlorophenoxyacetic acid were inactive.

When the ortho and para positions were substituted with chlorine or a methyl group making 2,4-dichlorophenoxyacetic or 2,4-dimethylphenoxyacetic acids, the compounds were active for cell elongation and for modification of leaves. The higher homologs, however, were different. α-(2,4-Dichlorophenoxy) propionic and butyric acid were not active for modification of organs while the corresponding methyl-substituted compounds were active. This peculiarity is illustrated in table 4.

The formative influence of 2,4-dichlorophenoxyacetic acid is illustrated in figure 1 B and C. When the *Nicandra physalodes* plant on the right was ap-

TABLE 2. The Dependence for Activity upon the Position of Substituted Groups



proximately five inches high, it was sprayed with a water solution containing 12.5 mg. of the chemical per liter of water. All new growth thereafter produced modified organs. The change in the pattern of leaves can be seen by comparing the three old leaves near the base of the stem which were nearly mature when the plant was treated with those of the new growth. Also the flowers of the new growth were modified as shown in figure 1 B and C. Stems and leaves sometimes became fasciated and flowers appeared to arise from leaves (Fig. 1 C). Calyx tubes often remain closed and prevented the corolla from emerging. The veins of the leaves often crowded toward the midrib making a narrow leaf with only a ruffle of blade around the edge. The veins became nearly transparent and the plants appeared to have a virus disease.

Substituted benzoic acids. Considered from the standpoint of a plant growth substance, benzoic acid is an inactive compound. When, however, the nucleus is substituted, the molecule may be activated. The degree of activity depends upon the kind, number, and the position of substituted groups. Amino, nitro,

TABLE 3. Molecular Configuration and Comparative Activity of Phenoxy and Substituted Phenoxy Compounds

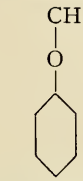
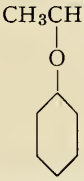
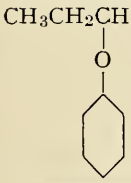
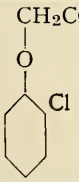
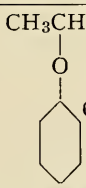
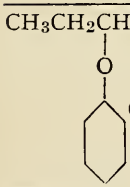
Substances	Cell elongation (epinasty) threshold conc. mg./g.	Modification of tomato leaves threshold conc. mg./g.
 Phenoxyacetic acid	20	Inactive
 α—(Phenoxy)propionic acid	5	5
 α—(Phenoxy)-*n*-butyric acid	5	5
 *o*-Chlorophenoxyacetic acid	1	0.25
 α—(2-Chlorophenoxy)propionic acid	1	Inactive
 α—(2-Chlorophenoxy)-*n*-butyric acid	1	Inactive

TABLE 3. (*Continued*)

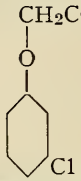
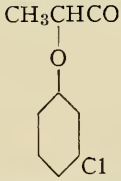
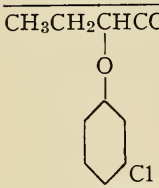
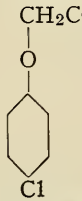
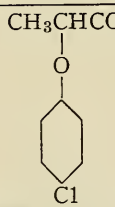
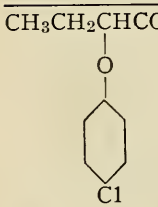
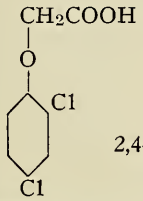
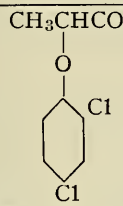
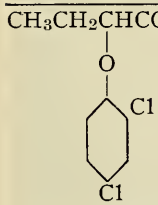
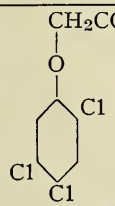
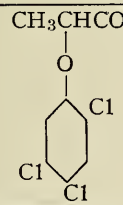
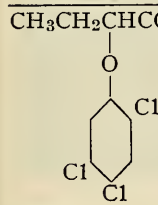
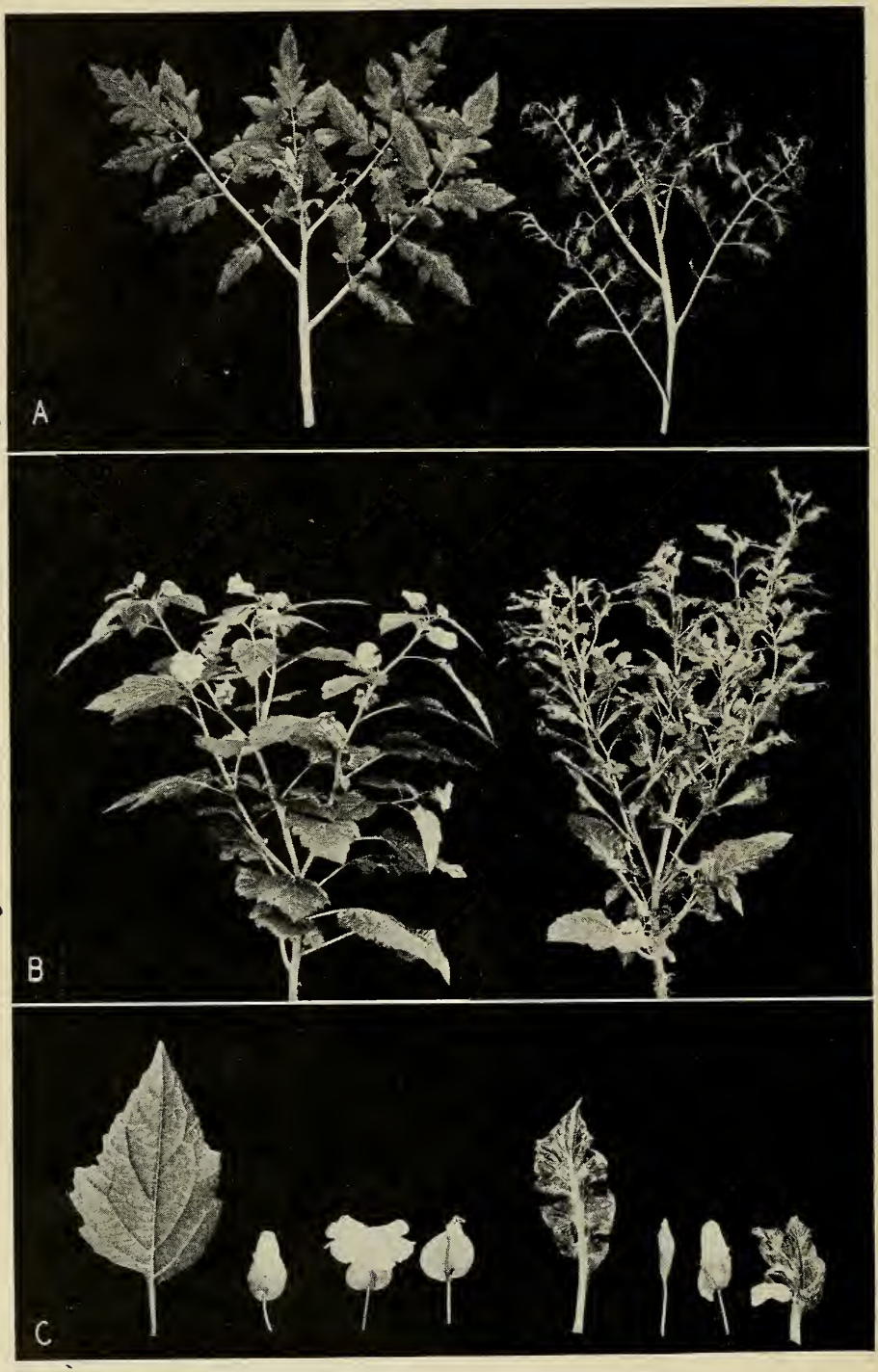
Substances	Cell elongation (epinasty) threshold conc. mg./g.	Modification of tomato leaves threshold conc. mg./g.
 *m*-Chlorophenoxyacetic acid	0.5	Inactive
 α—(3-Chlorophenoxy) propionic acid	0.5	Inactive
 α—(3-Chlorophenoxy)-*n*-butyric acid	0.5	Inactive
 *p*-Chlorophenoxyacetic acid	0.25	0.06
 α—(4-Chlorophenoxy) propionic acid	0.5	Inactive
 α—(4-Chlorophenoxy)-*n*-butyric acid	1	Inactive

TABLE 3. (*Concluded*)

Substances	Cell elongation (epinasty) threshold conc. mg./g.	Modification of tomato leaves threshold conc. mg./g.
 2,4-Dichlorophenoxyacetic acid	0.015	0.003
 α—(2,4-Dichlorophenoxy)propionic acid	0.5	Inactive
 α—(2,4-Dichlorophenoxy)-*n*-butyric acid	0.5	Inactive
 2,4,5-Trichlorophenoxyacetic acid	0.06	Inactive
 α—(2,4,5-Trichlorophenoxy) propionic acid	0.03	Inactive
 α—(2,4,5-Trichlorophenoxy)-*n*-butyric acid	0.1	Inactive


A
B
C

and halogen groups appear to be the most important. Some substituted benzoic acids have a pronounced formative influence on plants but have little or no effect on cell elongation. One compound of the group, however, induced both cell elongation and modification of leaves (3, 7). Table 5 shows a list of active and inactive compounds.

Positions 2, 3, and 5 in the nucleus appeared to be the most important for substitutions. For example, 2,3,5-triiodobenzoic acid and 2-chloro-3,5-diiodobenzoic acid had the most pronounced formative influence of any of the compounds listed. In addition to modifying the pattern of leaves, they influence flowering habit and correlation of organs (8). One to 5 mg. of triiodobenzoic acid added to the soil of a 4-inch pot in which a tomato plant was growing was sufficient to cause modification of growth of the stem, leaves, and flowers, to cause axillary buds to grow flower clusters instead of the normal leafy shoots, and to induce the terminal bud to terminate with a flower cluster instead of continuing with a leafy shoot (Fig. 2 A and B). Similar results were obtained by other methods of applying the chemical. It is effective as a lanolin preparation (1 to 10 mg./g. of lanolin), as a vapor applied in a closed container, and as a water solution applied as a spray (25 to 100 mg./l.).

The results obtained with 2-chloro-3,5-diiodobenzoic acid were similar to but even more striking than those described for triiodobenzoic acid. Both compounds caused the terminal bud and axillary buds of tomatoes to grow flower clusters instead of leafy shoots, but the individual flowers were different. Those which grew under the influence of 2-chloro-3,5-diiodobenzoic acid were small with inconspicuous petals and sepals supported with an abnormally stout peduncle (Fig. 3 A). As the chemical influence became weaker and the plants began to recover, large single flowers instead of clusters were produced irregularly along the stem. The small flowers did not set fruit but the large ones functioned as normal flowers (Fig. 3 A and B).

Another active substituted benzoic acid, 2-bromo-3-nitrobenzoic acid, is of special interest since it caused both cell elongation (epinasty) and modified leaves of tomato plants (7). It was not as active for cell elongation as some of the phenoxy compounds but had a pronounced formative influence on growth. 2-Chloro-5-nitrobenzoic acid has a formative influence but does not cause cell elongation. Judging from active benzoic acids listed in table 5 it would appear

Explanation of figure 1

Modification of organs induced with substituted phenoxy compounds. A. Tomato shoots: left, control; right, response to spray with solution of β-(2,4,6-trichlorophenoxy)-β'-chlorodiethyl ether. B. *Nicandra* plants: left, control; right, modifications induced with 2,4-dichlorophenoxyacetic acid (12.5 mg./l.). Solution applied at tip with nasal atomizer when plant was 5 inches in height. Note non-modified leaves at base which were present when treated. C. Enlarged leaves, buds, and flowers taken from plants in B.

TABLE 4. Variation in Activity According to the Position of Chloro and Methyl Substituted Groups

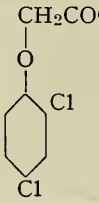
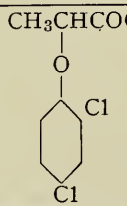
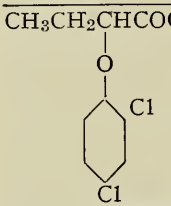
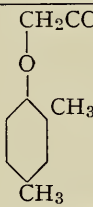
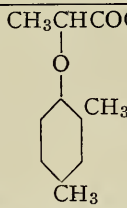
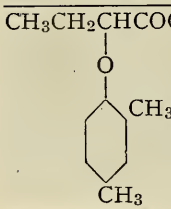
Substances	Cell elongation	Formative effects
 2,4-Dichlorophenoxyacetic acid	Active	Active
 α—2,4-Dichlorophenoxy)propionic acid	Active	Inactive
 α-(2,4-Dichlorophenoxy)-*n*-butyric acid	Active	Inactive
 2,4-Dimethylphenoxyacetic acid	Active	Active
 α—(2,4-Dimethylphenoxy)propionic acid	Active	Active
 α—(2,4-Dimethylphenoxy)-*n*-butyric acid	Active	Active

Fig. 2. Formative influence of 2,3,5-triiodobenzoic acid on tomato plants. A. Left, control; right, treated with 2 mg. of the chemical in 50 cc. of water applied to the soil when the plant was approximately 5 inches in height. B. Left, control; middle and right, terminal shoots showing modified flowering habit and correlation of organs after the main shoot had been treated with triiodobenzoic acid in lanolin (5 mg./g. [middle], and 10 mg./g.). Photographs taken 30 days after treatment.

TABLE 5. Molecular Configuration of Some Active and Inactive Benzoic Acids

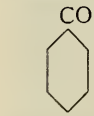
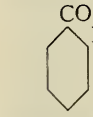
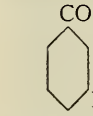
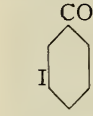
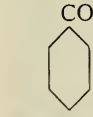
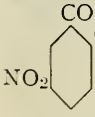
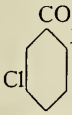
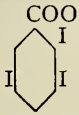
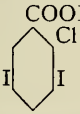
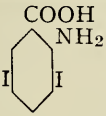
Substances	Cell elongation	Formative effects
 Benzoic acid	Inactive	Inactive
 2-Iodobenzoic acid	Inactive	Inactive
 3-Iodobenzoic acid	Inactive	Inactive
 4-Iodobenzoic acid	Inactive	Inactive
 2,4-Diiodobenzoic acid	Inactive	Inactive
 3,5-Diiodobenzoic acid	Inactive	Active
 2-Bromo-3-nitrobenzoic acid	Active	Active
 2-Chloro-5-nitrobenzoic acid	Inactive	Active

TABLE 5. (*Concluded*)

Substances	Cell elongation	Formative effects
 2-Amino-5-chlorobenzoic acid	Inactive	Inactive
 2,3,5-Triiodobenzoic acid	Inactive	Active
 2-Chloro-3,5-diiodobenzoic acid	Inactive	Active
 2-Amino-3,5-diiodobenzoic acid	Inactive	Inactive

that activity is dependent upon substitutions in the 2, 3, and 5 positions. None of the mono-substituted benzoic acids were active. Also 2,3,5-triiodobenzoic acid was more active than 3,5-diiodobenzoic acid. There are many possibilities for substituting a given radical and combinations of various groups in the nucleus of benzoic acids. Comparative activity cannot be predicted from the appearance of structural formulae. They must be synthesized and tested for comparative degrees of activity. That is to say, at the present time activity can be determined only by actual biological tests.

DISCUSSION

Formative influences described under the heading of "Results" are comparatively new in the study of growth substances. The meaning of the word "formative" could be extended to include local cell elongation and other short time responses which do not involve modification of size, shape, or pattern of organs produced by new growth. As intended in this paper, "formative" involves the growth of new organs immediately following the application of the active substance. The result is a systemic effect rather than local. It could be compared to the effect of a systemic virus disease in contrast to a local fungus disease. In fact the responses induced by some of the active compounds have been mistaken for virus diseases. As the character of the responses varies with


A
B

different strains of virus so do they vary with the different active growth substances. A characteristic virus-like type of response is illustrated in figure 4 for three different compounds applied to two different species of plants. The chemically induced responses have the characteristic modifications of leaves showing clearing of the veins, irregular shape, light and dark portions of tissue, etc. The present results appear to lend support to the claim that virus diseases result from natural chemical influences.

The mechanism in the plant through which these chemicals act is not well understood. It is fairly certain that living protoplasm has many potentialities for expressing itself and that environment determines which of these can develop. The so-called "normal" characters of a plant are but a partial expression of the range of possibilities of which the protoplasm is capable. Natural variations in the pattern of leaves of certain species of plants growing in different environments lend support to this theory. The influences which regulate growth must deal with undifferentiated meristems made up of uniform cells and in some way cause them to give rise to specialized cells which in turn give rise to new tissues and new organs of plants. Sinnott (2) is of the opinion that the cytoplasm plays an important rôle in "the *construction* of a pattern." It seems reasonable to assume that modified organs result from the influence of the chemicals acting upon the cytoplasm rather than upon the more stable nucleus. Each chemical constitutes a different environment and, therefore, permits different potentialities of the protoplasm to develop. This, at best, is only an assumption but may in time help us to interpret the qualitative differences in responses resulting from treatment with different growth substances.

The molecular configuration as a whole rather than any part of the molecule appears to determine physiological activity. A slight shift in the position of a substituted group, a change from chlorine to an amino group, or a shift in the linkage of the chain to the nucleus may activate or inactivate a molecule.

In addition to the exact nature of the molecule, the constitution of the receptor tissue in the plant is important. First, the genetic constitution of the tissue plays an important part, and second, the location in the organ and the age of the tissue are determining factors.

Explanation of figure 3

Formative influence of 2-chloro-3,5-diiodobenzoic acid. A. Left, control; middle, terminal portion of tomato plant after stem had been treated with a lanolin preparation (20 mg./g.). Note miniature flower. Right, two abnormally large individual flowers (instead of clusters) formed after one axillary shoot began to recover from the effects of the chemical influence. B. Left, control; right, terminal portion of the plant which had been given soil treatment of 2-chloro-3,5-diiodobenzoic acid (4 mg. per pot applied in 50 cc. of water). Note abnormally small flowers on 2 clusters and recovery of axillary shoot with abnormal flower cluster.

Though in the same family group, tomato and potato tissues do not respond alike to a given substance, due, perhaps to the difference in their genetic constitution. Apple and lilac stem cuttings can be induced through chemical treatment to produce adventitious roots in the spring of the year but not in autumn or winter. Though the tissue is receptive at an early age, the capacity to respond to the chemicals is soon lost.

Fig. 4. Shoots and leaves showing formative influence of growth substances. A. *Datura stramonium:* left, control; middle, sprayed with a solution of 2,4-dibromophenoxyacetic acid (50 mg./1.); right, sprayed with a solution of *p*-aminophenoxyacetic acid (500 mg./1.). B. Cucumber (*Cucumis sativus*) leaves: left, control leaf; right, 4 leaves taken from one plant after the terminal bud had been sprayed with a solution of β-naphthoxyacetic acid (100 mg./1.).

With still other species young tissue does not respond to chemical treatment whereas older tissue is susceptible. Many other illustrations could be given to indicate that there are complex internal and external influences playing upon the living protoplasm and that the sum total of these regulates the growth and development of the plant.

SUMMARY

"Formative influence" as used in this report is defined and distinguished from other hormone-like influences.

For the study of formative influences three groups of compounds, β-naphthoxy, substituted phenoxy derivatives of the lower fatty acids, and substituted benzoic acids, stand out above all others. Substitutions in the nucleus of the molecule may be made with halogens, amino, nitro, or methyl groups. These may be used separately or in combination. Physiological activity depends upon the kind, number, and position of the substituted groups. The molecular configuration as a whole rather than any one part of the molecule appeared to determine the activity.

Characteristic responses induced with these growth regulators are illustrated in four different figures. Attention is called to the similarities between responses induced with synthetic growth regulators and naturally occurring virus diseases.

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